and AO1. Results are virtually identical when the most likely haplotype explanation is assigned to ambiguous families (see table B in the online-only supplemental material). As is obvious from table 2, the agreement between nominal and true type I error rate is disastrous in the presence of genotyping errors. Even quite small probabilities of genotyping errors lead to a dramatic inflation of the type I error. For fixed values of ε, the extent of this inflation increases with increasing sample size (N), as can be seen by comparing the second and third row in table 2. For a large sample size of $N = 1{,}000$ family trios, an error probability of $\varepsilon = 0.1\%$ is sufficient to falsely reject the null hypothesis at $\alpha = 0.05$ in almost every sixth study. For small values of N and large values of ε, the inflation of type I error is slightly less pronounced for EO2 than for EO1, which is explained by noting that EO2 leads to a decrease of the sample size used for the analysis. At first sight, it may be surprising that no essential decrease of the inflation of type I error is obtained by employing EO3. However, correcting genotypes leading to MIs does not affect errors in the nontransmitted haplotypes.

What are possible limitations of our simulation study? We assume a specific haplotype structure in the population, such that only 29 different haplotypes are present. Indeed, we conjecture that with larger haplotype diversity, the effect of genotyping errors on the type I error rate of the HS-TDT will be less pronounced than in the example considered here. On the other hand, however, it does not seem very realistic to expect that the HS-TDT will have substantial power to detect a disease locus in a region in which the markers are in complete or nearly complete linkage equilibrium in the population. Thus, although our example describes a specific situation, it does not seem to be unrealistic for the genetic structure of a region for which the HS-TDT may have a good chance of detecting a disease locus. A second possible limitation is that we employed a quite simple error model that assumes the independence of genotyping errors from factors such as marker locus, true allele, etc. However, we see no reason why the behavior of the type I error rate of the HS-TDT should be qualitatively different for more complex models of genotyping errors. Additionally, we are convinced that the range 0.1%–1% for the probability (ε) of a genotyping error considered here is not too pessimistic for currently available methods of high-throughput genotyping.

In summary, we have shown that the correctness of genotypes is crucial for obtaining meaningful results by the HS-TDT. We have also demonstrated that the retyping of only those marker loci that show MIs within a family is useless. A more extreme approach is to genotype all marker loci in all families in duplicate, which is very expensive and certainly not very popular with geneticists responsible for generating genotypes. However, unless extreme care is taken to guarantee the integrity of the data analyzed by the HS-TDT, this interesting and appealing method has the potential of becoming a mighty tool for the enlargement of the heap of false-positive association results in human genetics.

Acknowledgments

This work was supported by grant Kn378/1 (Project D1 of FOR 423) from the Deutsche Forschungsgemeinschaft.

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Electronic-Database Information

The URL for data presented herein is as follows:

FAMHAP: Haplotype Frequency Estimation, http://www.uni-bonn.de/~umt70e/becker.html

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0002-9297/2004/7403-0031$15.00

Am. J. Hum. Genet. 74:591–593, 2004

Reply to Knapp and Becker

To the Editor:

Knapp and Becker (2004 [in this issue]) have argued that genotyping errors may lead to an inflated type I

Table 1

Parameters and Results of Simulation Study of Type I Error Rate of HS-TDT in the Presence of Genotyping Error

	Parameters			Type I Error Rate for			
				EO2		EO3	
Similarity Measure	No. of Children/ Family	No. of Nuclear Families/ Sample	Typing Error Rate (ϵ)	$\alpha = .05$[a]	$\alpha = .01$[a]	$\alpha = .05$[a]	$\alpha = .01$[a]
Original:	1	100	.01	.457	.227	.364	.147
	1	100	.005	.228	.08	.193	.075
	1	200	.005	.364	.147	.315	.120
	3	100	.01	.053	.012	.06	.016
	3	100	.005	.056	.013	.046	.011
	3	200	.005	.044	.008	.053	.006
New:	1	100	.01	.117	.037	.092	.019
	1	100	.005	.079	.016	.073	.014
	1	200	.005	.081	.016	.101	.029
	3	100	.01	.059	.016	.042	.006
	3	100	.005	.059	.015	.043	.009
	3	200	.005	.047	.010	.045	.003

Note.—The "original similarity measure" refers to the one used by Zhang et al. (2003). Simulation studies were based on 1,000 replicated samples.

[a] α = nominal type I error rate.

error rate for the haplotype-sharing transmission/disequilibrium test (HS-TDT) that we proposed (Zhang et al. 2003). The reason is that transmitted haplotypes are partially checked for genotyping errors by Mendelian inconsistency (MI), whereas there is no such checking at all for nontransmitted haplotypes. As a result of the unbalanced checking for genotyping errors, nontransmitted haplotypes appear less similar than transmitted haplotypes, which may lead to an inflated type I error rate for the HS-TDT. This is especially true for cases in which there is only one child per nuclear family. As noted by Gordon et al. (2001), the original TDT also has this problem. The HS-TDT that we proposed is applicable to any size of nuclear family and to different traits. To quantify the magnitude of type I error inflation of HS-TDT, Knapp and Becker (2004) performed a simulation study of nuclear families with one child. In fact, the magnitude of the type I error inflation caused by the unbalanced checking of the genotyping errors depends on the genotyping error rate as well as the following factors:

1. The number of children. If there is more than one child in the nuclear family, the genotyping errors in the haplotypes that do not transmit to the first child may be still detectable because these haplotypes may transmit to the other children. So, the inclusion of families with more than one child can reduce the type I error inflation.
2. The allele frequencies. A smaller minor allele frequency will lead to a larger probability of homozygous genotypes and, therefore, a larger probability of detectable genotyping errors (MI). Consequently, it will lead to larger type I error inflation (see table 3 of Gordon et al. 2001). For HS-TDT, a marker with a small minor allele frequency in the middle part of the haplotype has a bigger effect than a marker with a small minor allele frequency in the edge part of the haplotype.
3. The haplotype similarity measure.

We believe that the reasons for the high type I error rate of HS-TDT in Knapp and Becker's simulation studies are the following: (1) only families with one child were used; (2) the minor allele frequencies are small for the markers in the middle part of the haplotypes (for the total 19 markers, the minor allele frequencies from marker 7 to marker 16 are 0.16, 0.125, 0.143, 0.143, 0.11, 0.268, 0.089, 0.143, 0.143, and 0.036, respectively); and (3) the haplotype similarity measure that we proposed in Zhang et al. 2003 is not robust to genotyping errors. To compare the different haplotype similarity measures, we propose another measure (called "new similarity measure") as follows. For two haplotypes, H and h, let H_i (h_i) denote the allele of the haplotype H (h) at marker i. To find the similarity measure of the two haplotypes around marker i, we compare alleles of the two haplotypes in the right-hand markers, beginning with marker $i + 1$, until marker $i + r$ satisfies $H_{i+r} \neq h_{i+r}$ and either $H_{i+r+1} \neq h_{i+r+1}$ or $H_{i+r+2} \neq h_{i+r+2}$. Then, similarly, we compare alleles of the two haplotypes in the left-hand markers, beginning with

marker $i-1$, until marker $i-l$ satisfies $H_{i-l} \neq h_{i-l}$ and either $H_{i-l-1} \neq h_{i-l-1}$ or $H_{i-l-2} \neq h_{i-l-2}$. The new similarity measure is defined as the distance between marker $i-l$ and marker $i+r$. Note that a genotyping error that occurs at one marker but does not occur at the nearby markers will not affect the new similarity measure. The probability that genotyping errors will occur in several consecutive markers is very small. To compare the effect of the number of children and different haplotype similarity measures, we performed simulation studies in which we used the data and the error options EO2 and EO3 given by Knapp and Becker (2003). We did not use EO1 because our program automatically deletes the families with MI genotyping errors. The simulation results are summarized in table 1. This table reveals that, if there are three children in each of the nuclear families, a good agreement between the nominal and estimated type I error rate is evident for all the simulated samples. In the case of one child per family, the inflation of the type I error rate is greatly reduced by using the new similarity measure. We currently are investigating methods that are more robust to genotyping errors.

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